Egyptian Journal of Aquatic Research (2016) **42**, 289–299

HOSTED BY

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National Institute of Oceanography and Fisheries

Egyptian Journal of Aquatic Research

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FULL LENGTH ARTICLE

GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae

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Received 21 April 2016; revised 27 July 2016; accepted 28 July 2016
Available online 1 September 2016

KEYWORDS

Macroalgae;
Antifungal activity;
C. albicans;
GC/MS technique;
Palmitic acid;
Myristic acid

Abstract The antifungal activity and the chemical constituents of selected macroalgae collected from the Egyptian Mediterranean coast of Alexandria have been investigated. Agar well diffusion assay was used to determine the antifungal potential of the extractable matter against *Fusarium solani*, *Fusarium oxysporum*, *Tricoderma hamatum*, *Aspergillus flavipes* and *Candida albicans*. The ethyl acetate and methanolic extracts (ULE2 and ULM5) of *Ulva lactuca* obtained from Al Selsela exhibited the highest activity with (AI) = 1.05 ± 0.053 and 1.03 ± 0.052, respectively, compared with fluconazole. However, the methanolic extract of *U. lactuca* (ULM1) from Abu Qir Bay showed (AI) = 0.73 ± 0.037. This followed by methanolic extracts of *Pterocladia capillacea* (PCM1: AI = 0.70 ± 0.035 and *Ulva fasciata* (UFM1: AI = 0.69 ± 0.035). GC/MS analysis of ULM1 and ULM5 indicated the existence of different constituents revealing ecological impacts. The methanolic extract (UFM1) contains six major components including palmitic acid, methylester, trichloromethyloxirane, linolenic acid, ethylester, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 11-octadecenoic acid, methylester and 12,15-octadecadienoic acid, methylester. High percentages of palmitic acid, n-heptacosane, 2-methylhexadecan-1-ol, methoxy acetic acid, 2-tridecylester and myristic acid are found in the methanolic extract of *P. capillacea* (PCM1). Most of the identified components have been reported to possess antimicrobial activity that could be responsible for the antifungal potential reported in the present study.

Introduction

Marine environment is a good source of bioactive natural products exhibiting structural/chemical features that are not found in terrestrial natural compounds (Carter, 1996). Several marine organisms produce bioactive metabolites in response to

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Peer review under responsibility of National Institute of Oceanography and Fisheries.

http://dx.doi.org/10.1016/j.ejar.2016.07.003

ecological pressures (Ireland et al., 2000). They develop a chemical strategy for defense against other organisms in their environment and to ensure their survival (König et al., 1994). Numerous novel compounds have been isolated from marine organisms (Jha and Zi-rong, 2004; Arunkumar et al., 2010; D'Orazio et al., 2012; Mayer et al., 2013; Balakrishnan et al., 2014; Pal et al., 2014). Blunt et al. (2016) reported that approximately 25 700 new marine natural products (MNPs) have been discovered over the period 1965–2014 from 22 oceanic regions or countries. There have been extensive collections with 2358 compounds in the Mediterranean, Arabian Peninsula and Black sea regions. This high diversity has been the source of unique natural compounds used for various industrial developments such as pharmaceuticals, cosmetics and nutritional supplements (Suleria et al., 2015). Marine macroalgae have been widely recognized as producers of a broad range of biogenic compounds including polyunsaturated fatty acids, flavonoids, terpenoids, alkaloids, quinones, sterols, polyketides, phlorotannins, polysaccharide, glycerols, peptides and lipids (Al-Saif et al., 2014; El-Din and El-Ahwany, 2015) that have antimicrobial (Zbakh et al., 2012), antifouling (Bhadury and Wright, 2004), anti-inflammatory (Jaswir and Monsur, 2011), antiviral (Bouhlal et al., 2011), antioxidant (Devi et al., 2011), anticancer (Kim et al., 2011), anti-allergic (Na et al., 2005) and anticoagulant activities (Dayong et al., 2008; Kolanjinathan et al., 2014; Smit, 2004). For example, the green alga *Enteromorpha compressa* is rich in C_{18} PUFAs ± α-linolenic (18:3n-3) and octadecatetraenoic (18:4n-3) acids where α-linolenic acid is the main polyunsaturated acid (Khotimchenko et al., 2002) and the flavonoids content is 33.39 mg/g as reported by Sarojini et al. (2012).

In recent years, a significant number of reviews reported numerous investigations that have been carried on crude and purified compounds obtained from marine algae to evaluate their bioactive potentials (Mhadhebi et al., 2012; Balamurugan et al., 2013; Barbosa et al., 2014; Torres et al., 2014; Kolanjinathan et al., 2014; El-Din and El-Ahwany, 2015; Hamed et al., 2015; Blunt et al., 2016). The first antimicrobial substances produced by algae have been reported by Harder (1917). The variation in the production of antimicrobial substances by the same species might be due to ecology, sexual maturity or the stage of active growth (Pesando, 1990; Burkholder et al., 1960). The extracts of an alga collected from the same location are more effective than the same species collected from another location as reported by Arunkumar et al. (2010). Lee et al. (2010) studied the antifungal activity of *Ecklonia cava* brown algae against *Trichophyton rubrum*. A study on the antifungal activities of five species of *Laurencia* red algae against three strains of *Candida* spp. was carried out (Stein et al., 2011). Crude extracts of green, brown and red algae have been screened for the antifungal potential against standard dermatophytes strains (Guedes et al., 2012). Recently, screening of the antifungal activity from marine algae as prominent natural antibiotic was presented by Chowdhury et al. (2015) where new terpenoid derivatives from the red alga *Laurencia obtusa* considered as effective antifungal-antitumor agents (Alarif et al., 2015). Different analysis methods such as GC/MS, LC/MS, HPLC/DAD and CE/DAD (diode array detection) have been developed for the identification of the bioactive constituents (Gong et al., 2001). Mass selective detector spectral information in addition to retention time, peak height and peak area information enhances the identification of the components.

Several studies have been reported on the antibacterial activity of marine algae however, available information on their antifungal activity is limited. Consequently, the present investigation aimed to evaluate the antifungal activity of six Mediterranean macroalgae extracts, collected from the coastline of Alexandria, against five pathogenic fungi in order to discover potential antifungal metabolites. Qualitative identification of the most potential antifungal extracts of *Ulva lactuca*, *Ulva fasciata* and *Pterocladia capillacea* was performed using retention times and mass spectra in the GC/MS analysis.

Materials and methods

Samples collection

Six fresh seaweeds; *U. lactuca*, *U. fasciata*, *E. compressa*, *P. capillacea*, *Hypnea musciformis* and *Padina pavonica* were harvested at various sites along the Mediterranean Egyptian coast of Alexandria (Abu Qir Bay, Al Selsela and El-Anfoushy). After collection, the samples were rinsed with fresh water to remove associated epiphytes and debris. The cleaned algal materials were then air dried to dryness in the shade and ground into fine powder using electric grinder mixer.

Preparation of the algal extracts

Extraction of the bioactive algal extracts has been carried out using ethyl acetate and methanol as follows; the finely powdered algal material (100 g) was macerated separately in ethyl acetate (1.5 L) followed by methanol (1.5 L) at room temperature for a period of one week with regular shaking. After filtration, organic solvents were evaporated under vacuum at 45 °C to furnish dry ethyl acetate and methanolic extracts. The extraction procedure with each solvent was repeated once again to furnish ethyl acetate extracts as green residues while methanolic extracts were separated as green residues (ULM1, ULM5, UFM1, ECM, PCM1, HM1, and PPM1) or white precipitates (ULM3, ULM4), crystals (ULM7) or pale yellow liquids (ULM2, ULM6, UFM2, PCM2, HM2, PPM2). The crude extractable materials were then stored at -20 °C until use (Choudhury et al., 2005; Wefky et al., 2009; Shobier et al., 2010).

Test organisms

The test organisms used in this study (*Fusarium solani* AUMC 6448, *Fusarium oxysporum* AUMC 6449, *Tricoderma hamatum* AUMC 382, *Aspergillus flavipes* AUMC 6450 and *Candida albicans* ATCC 10231) were kindly provided by Assiut University Mycological Center, Faculty of Science. These fungal strains were subcultured onto potato-dextrose agar (PDA) supplemented with Rose Bengal for 5–7 days at 28–33 °C.

Antifungal assay

The *in vitro* antifungal analysis of the studied algal extracts was recorded in terms of a well cut technique (Bodet et al., 1985). Fungal colonies were diluted in sterilized water to 0.5

MacFarland scale turbidity standard (10^7 spores/ml suspension). Individually, each dermatophyte and *Candida* species were inoculated through swabbing on plates containing solid PDA. Four wells with 5 mm in diameter were punched for 100 µl of algal extracts (without dilution for liquid samples and at a concentration of 1 mg/ml for others). Afterward, plates were incubated at 28 °C for 7 days with dermatophytes and 33 °C for 48 h with *Candida* species and the results were determined from the presence or absence of growth and size of the inhibition zone.

Activity index

The activity index (AI) was used to compare the antimicrobial activity of the algal extracts against all fungal strains with that of the standard drug at the tested concentrations. It was calculated using the following equation (Egharevba et al., 2010)

$$\mathrm{AI} = \frac{\text{Mean of the extract inhibition zone diameter}}{\text{Mean of the standard antibiotic drug inhibition zone diameter}}$$

Microdilution assay

Microdilution assay was performed according to CLSI document M38-A2 (2002). The suspensions of each fungus were prepared with 5 ml of sterile distilled water. The suspensions were then diluted (1:50) in order to obtain the final inoculum concentration of 5×10^4 spores/ml. Potent algal extracts (100 µl) with different concentrations from 1 to 265 µg ml^{-1} were added in test tubes containing 3 ml potato-dextrose broth (PDB). Equal volume of cell suspensions (100 µl) was inoculated and incubated without agitation, for result interpretation. Control was included for each test, containing medium with 100 µl of antibiotics (positive control) and medium with 100 µl spores (negative control). The antifungal activities of the potent algal extracts were compared with reagent-grade powder antibiotics; miconazole, fluconazole and itraconazole at 100 µg ml^{-1} while dimethyl sulfoxide (DMSO) was used as negative control. Each assay was carried out in duplicate and the minimum inhibitory concentration (MIC) of algal extracts was determined by visual inspection of each tube through the evaluation of the fungal growth inhibition using growth as control. MICs were determined by choosing the lowest concentration of the algal extract preventing fungal spores (da Silva Barros et al., 2007).

Gas chromatography/Mass spectrometry (GC/MS) analysis

GC/MS analysis was carried out using Gas Hewlett Packard HP-5890 series II equipped with split/splitless injector and a capillary column (30 m, 0.25 mm, 0.25 µm) fused with phenyl polysilphenylene siloxane. The injector and detector temperatures were set at 280 and 300 °C, respectively, and the oven temperature was kept at 80 °C for 1 min, rose to 300 °C at 20 °C/min. Helium was used as carrier gas at a constant flow of 1.0 ml/min. A volume of 2 µl was injected in the splitless mode and the purge time was 1 min. The MS (Hewlett–Packard 5889B MS Engine) with selected ion monitoring (SIM) was used. The mass spectrometer was operated at 70 eV and scan fragments from 50 to 650 m/z. Peak identification of crude algal extracts was performed based on comparing the obtained mass spectra with those available in NIST library.

Statistical analysis

Origin Lab 6 was used to find out the significant difference at 5% level and analyze the activity indices of each algal extract.

Results and discussion

Antifungal potential

In the present work, different extractable materials from marine algae collected from the Egyptian Mediterranean coastline of Alexandria have the ability to produce bioactive compounds with potential therapeutic interest. The methods used to evaluate the antifungal activity were agar diffusion and MIC techniques due to their low cost and simple implementation (Ibtissam et al., 2009). The combination of different methods provides a significant platform to discover new antifungal drugs for dermatophytosis and mycosis disease treatment (Peres et al., 2010).

The highest antifungal activities against all tested fungi were exerted by both ethyl acetate (ULE2) and methanolic (ULM5) extracts of *U. lactuca*, collected from Al Selsela, with IZ ranging from 18 to 32 mm, average zone of inhibition 23.2 and 22.6 mm and activity index (AI = 1.05 ± 0.053 and 1.03 ± 0.052, respectively) (Table 1, Figs. 1 and 2). However, the methanolic extract of *U. lactuca* (ULM1) obtained from Abu Qir Bay exhibited an (AI) value at 0.73 ± 0.037. The methanolic extracts of *U. fasciata* (UFM1) and *P. capillacea* (PCM1) showed an average antifungal activity (AVG) of 15.2 and 15.4 mm; (AI) = 0.69 ± 0.035 and 0.70 ± 0.035, respectively. Lower antifungal activities were detected by the remaining extracts that possessed AI values between 0.24 ± 0.012 and 0.55 ± 0.035 except PPM1 in which, the minimum of three fungal strains must be susceptible to achieve higher antifungal activities for comparison (Fig. 2). Similarly, the antifungal activity of different green algal species studied by Abedin and Taha (2008) against *Aspergillus niger* and *Aspergillus flavus* ranged from 20 to 35 mm. Ertürk and Taş (2011) mentioned that *Ulva rigida* extracts showed activity against *A. niger* and *C. albicans* at 12 mm. Chloroform and ethyl acetate extracts obtained from marine brown algae showed antifungal effect against *C. albicans* that ranged from 9 to 25 mm as reported by Mhadhebi et al. (2012). However, no activity was observed using different *U. lactuca* extractable solvents (Guedes et al., 2012). Nine organic fractions of hexane, ethyl acetate and methanolic extracts from the red alga *Bostrychia tenella* have been reported to display strong fungal growth inhibition in *Cladosporium* sp. as an evaluated strain (de Felício et al., 2010).

Since microdilution method is the best technique mainly, due to the sensitivity and minimal amount of reagents, thus applying 2 µg ml^{-1} of *U. lactuca* extract (ULE2) exhibited the maximum activity against *T. hamatum* compared with fluconazole and itraconazol which had activities at 4 µg ml^{-1} and 8 µg ml^{-1}, respectively. This was followed by the effect of *U. lactuca* methanolic extract (ULM5) against *A. flavipes* and *F. oxysporum* with MIC = 4 µg ml^{-1}. Almost all fungal strains were susceptible to *U. lactuca* methanolic extract (ULM1) by 8 µg ml^{-1} where both fluconazole and itraconazol have the same effect. Higher MIC values were detected using *U. fasciata* extract (UFM1) since 128 µg ml^{-1} was enough to

Table 1 Diameter of the inhibition zones (mm) of crude extracts against different fungal species.

Species	Extract	Sample Code	Inhibition zone diameter (mm)					
			F. solani AUMC 6448	*F. oxysporum* AUMC 6449	*T. hamatum* AUMC 382	*A. flavipes* AUMC 6450	*C. albicans* ATCC 10231	AVG
U. lactuca (Abu Qir Bay)	Ethyl acetate	ULE1	0	10	15	15	12	10.4
	Methanol	ULM1	12	23	23	22	0	16
		ULM2	11	15	0	0	0	5.2
		ULM3	0	12	0	10	11	6.6
		ULM4	0	20	0	15	10	9
U. lactuca (Al Selsela)	Ethyl acetate	ULE2	18	18	25	32	23	23.2
	Methanol	ULM5	18	25	25	25	20	22.6
		ULM6	13	0	15	15	0	8.6
		ULM7	0	0	10	13	12	7
U. fasciata (El-Anfoushy)	Ethyl acetate	UFE	0	0	0	15	15	6
	Methanol	UFM1	18	18	10	20	10	15.2
		UFM2	13	15	12	0	0	8
E. compressa (Abu Qir Bay)	Methanol	ECM	15	0	0	22	13	10
P. capillacea (Abu Qir Bay)	Methanol	PCM1	15	15	15	15	17	15.4
		PCM2	10	15	0	0	15	8
H. musciformis (Abu Qir Bay)	Methanol	HM1	12	15	14	0	12	10.6
		HM2	10	0	13	25	12	12
P. pavonica (Abu Qir Bay)	Methanol	PPM1	10	18	25	10	14	15.4
		PPM2	10	0	10	0	12	6.4
Miconazole (MCZ)			20	20	20	25	19	20.8
Fluconazole (FLC)			22	20	23	25	20	22
Itraconazole (ITC)			20	22	20	22	21	21

AVG: average of inhibition zone.

cease the growth of *A. flavipes* and *C. albicans* (Table 2). A previous study by Ertürk and Taş (2011) indicated that the antifungal activity of *U. rigida* extracts against *A. niger* and *C. albicans* was at MIC more than 10 μg ml^{-1}. Screening for the antifungal activities of Brazilian seaweed extracts against *C. albicans* (ATCC 10231) showed a range of 65–303 μg ml^{-1} (Stein et al., 2011). Dichloromethane and ethanol extracts from *H. musciformis* had MIC at 8 μg ml^{-1} against *C. albicans*. *Padina gymnospora* chloroform extract demonstrated MIC at 16 μg ml^{-1} against *Candida parapsilosis* and *Candida krusei* (Guedes et al., 2012). Guedes et al. (2012) reported that *U. lactuca* aqueous extract exhibited MIC at 5 μg ml^{-1} against *T. rubrum* and *Epidermophyton floccosum*. The antifungal potential from the marine red alga *B. tenella* showed MIC values ranging from 16 to 19 μg ml^{-1} (de Felício et al., 2010).

GC/MS analysis of the most effective extracts

Many species of macroalgae contain useful constituents such as myristic acid, palmitic acid, stearic acid (Agoramoorthy et al., 2007; Balamurugan et al., 2013), phenols, indoles, acetogenins, terpenes, labdane diterpenes, brominated hydroquinones, tropodithietic acid and phlorotannins (Ibañez et al., 2012; Samarakoon and Jeon, 2012) which may produce antibiosis against fungi which cause the antimicrobial activity of algae. Bergasson et al. (2011) reported that lipids block microbes by distracting the cellular membrane of bacteria, fungi and yeasts. Balamurugan et al. (2013) isolated n-hexadecanoic acid, tetradecanoic acid, oleic acid, 9-octadecenoic acid, 6-octadecenoic acid, hexadecanoic acid, ethyl ester, ethyl tridecanoate and octadecanoic acid from the ethanolic extract *of H. musciformis*. Usha and Maria Victorial Rani (2015) identified several compounds in the methanolic extract of *P. pavonica* including myristic acid, palmitic acid, linoleic acid, myristic acid ester, palmitic acid ester and oleic acid ester. Terpenes and sterols are present in high amount in this alga where 3-furoic acid and phytol are the main components of terpenes (El Shoubaky and Salem, 2014-2015).

In the present investigation, the CG/MS analysis of *U. lactuca*, *U. fasciata* and *P. capillacea* crude extracts; ULE2, ULM1, ULM5, UFM1 and PCM1 showed a mixture of various components. The total number of the main peaks observed for *U. lactuca* collected from Al Selsela was 11 peaks for ethyl acetate (ULE2) and 14 peaks for methanolic (ULM5) extracts (Figs. 3 and 4). However, the methanolic extract of *U. lactuca* obtained from Abu Qir Bay (ULM1) exhibited 13 peaks (Fig. 5). The methanolic extract of *U. fasciata* harvested from El-Anfoushy (UFM1) possessed 17 peaks (Fig. 6) while, *P. capillacea* methanolic extract (PCM1) collected from Abu

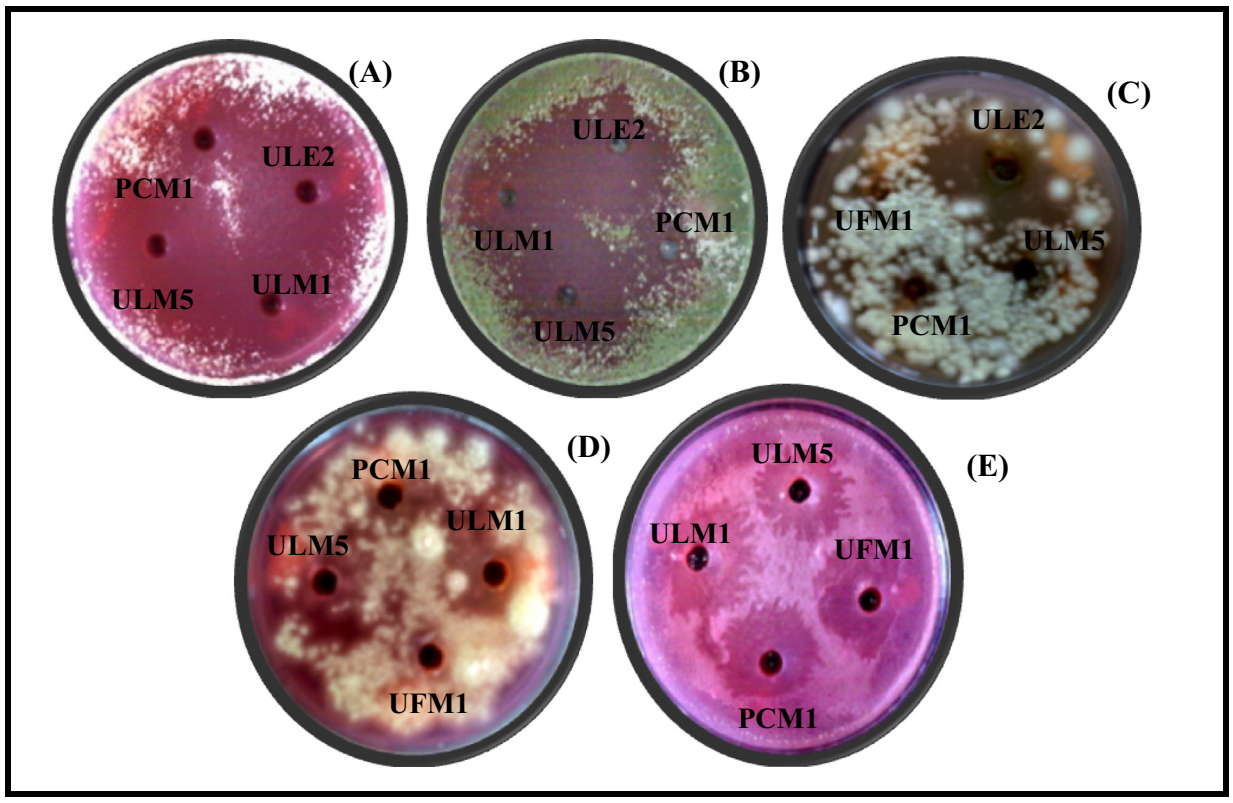

Figure 1 Antifungal activity of *U. lactuca*, *U. fasciata* and *P. capillacea* extracts (ULM1, ULE2, ULM5, UFM1 and PCM1) against *A. flavipes* (A), *F. solani* (B), *F. oxysporum* (C), *T. hamatum* (D) and *C. albicans* (E).

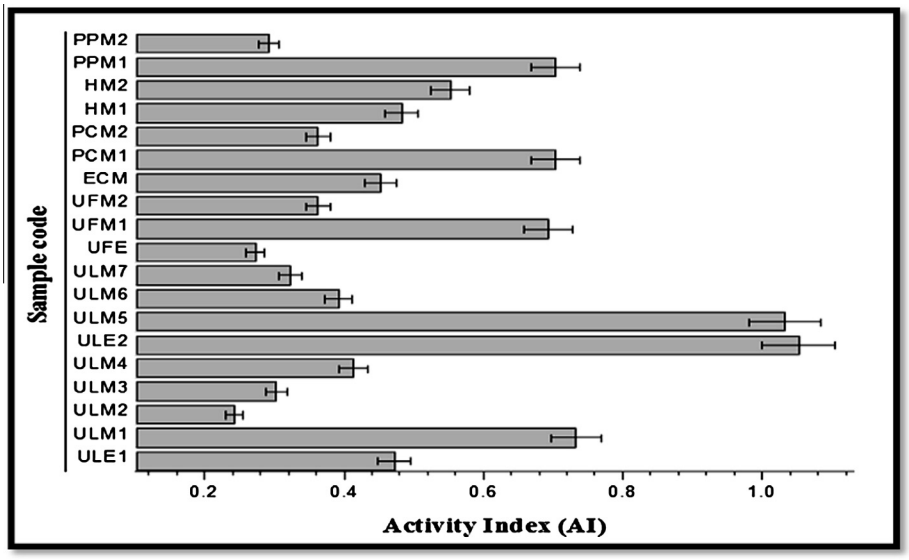

Figure 2 Activity indices of crude algal extracts against test fungal strains. Bars are expressed as mean ± SE; $n = 5$ (Origin Lab. 6).

Qir Bay gave rise to 15 peaks (Fig. 7). The components were identified and compared with previously isolated compounds. Most of these components have been found to possess antimicrobial, antioxidant, antiinflammatory, antitumor and anticancer activities (Smit, 2004; Mayer and Hamann, 2004; Mayer and Lehmann, 2001; Flora and Maria Victorial Rani, 2013; El Shoubaky and Salem, 2014-2015; Usha and Maria Victorial Rani, 2015). Results indicated that seven phytocompounds were characterized and identified in the *U. lactuca* ethyl acetate extract (ULE2) as shown in Table 3. The main chemical constituents found in high percentages are dichloroacetic acid, heptadecyl ester (retention time RT = 7.91 min) followed by (9Z)-9,17-octadecadienal (RT = 8.79 min), methyl 14-methylheptadecanoate (RT = 9.55 min), and di-n-

Table 2 Minimum inhibitory concentration (MIC) of crude algal extracts against test fungal strains compared with standard antibiotics used as antifungal drugs.

Algal extracts	MIC (μg/ml)				
	A. flavipes AUMC 6450	*F. solani* AUMC 6448	*F. oxysporum* AUMC 6449	*T. hamatum* AUMC 382	*C. albicans* ATCC 10231
U. lactuca (ULE2)	4	16	16	2	8
U. lactuca (ULM1)	8	64	8	8	16
P. capillacea (PCM1)	32	32	16	32	16
U. fasciata (UFM1)	128	16	16	16	128
U. lactuca (ULM5)	4	16	4	4	16
Miconazole	16	16	16	4	16
Fluconazole	8	8	16	4	16
Itraconazole	16	16	8	8	8

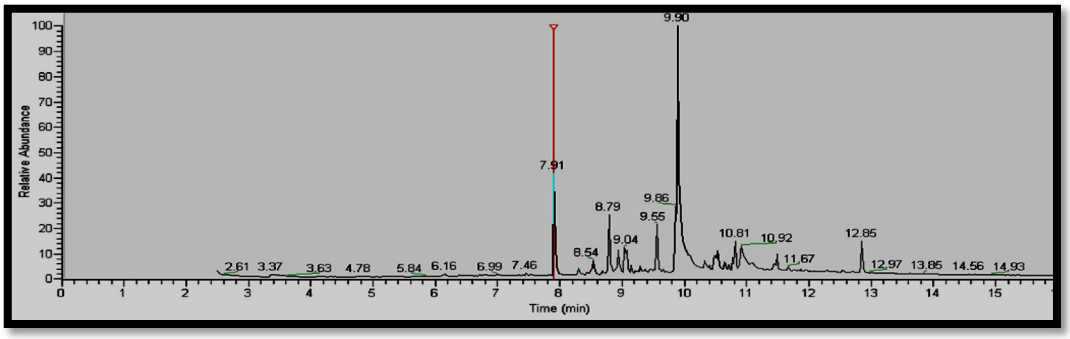

Figure 3 GC/MS chromatogram of *U. lactuca* ethyl acetate extract (ULE2) collected from Al Selsela.

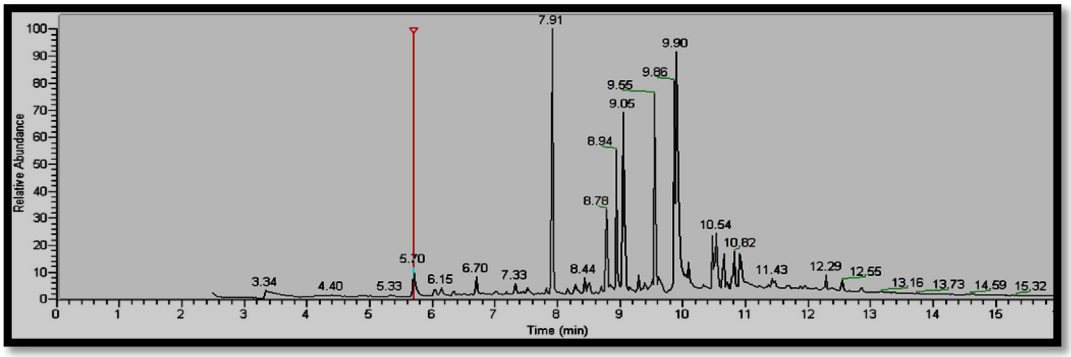

Figure 4 GC/MS chromatogram of *U. lactuca* methanolic extract (ULM5) collected from Al Selsela.

octylphthalate (RT = 12.85 min). For methanolic extract (ULM5) of the same alga, eleven components were identified and the most abundance five constituents were 1-heptadecanol (RT = 7.91 min) followed by palmitic acid, ethylester (RT = 9.86 min), oleic acid (RT = 9.55 min), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (RT = 9.05 min) and 3-icosyne (RT = 8.78 min) (Table 4). However, GC/MS analysis of the methanolic extract of *U. lactuca* (ULM1) revealed that nine components were identified with retention times as presented in Table 5. The main identified constituents found in high amounts are phthalic acid, isodecyloctylester (RT = 12.85 min), myristyl chloride (RT = 7.94 min) and trichloroethylene (RT = 5.69 min). Previous phytochemical investigation on *U. lactuca*, collected from the Alexandria coast, led to the isolation of (*E*)-6-octadecen-5-ol and (*E*)-6-heptacosen-5-one as new compounds along with palmitic acid,

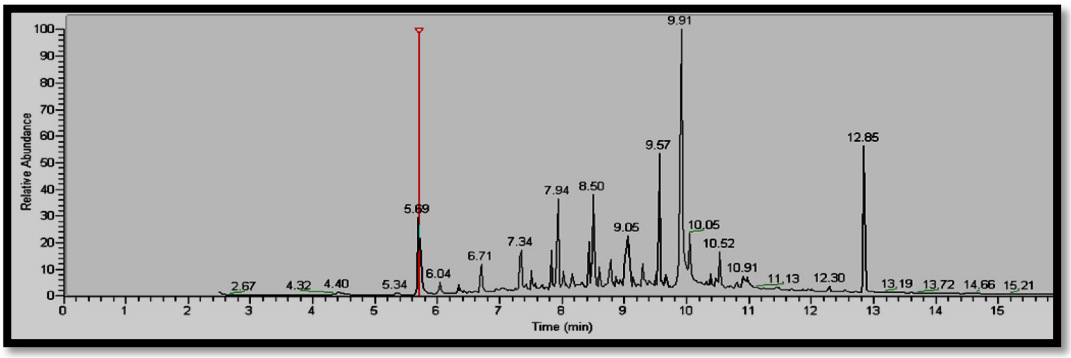

Figure 5 GC/MS chromatogram of *U. lactuca* methanolic extract (ULM1) collected from Abu Qir Bay.

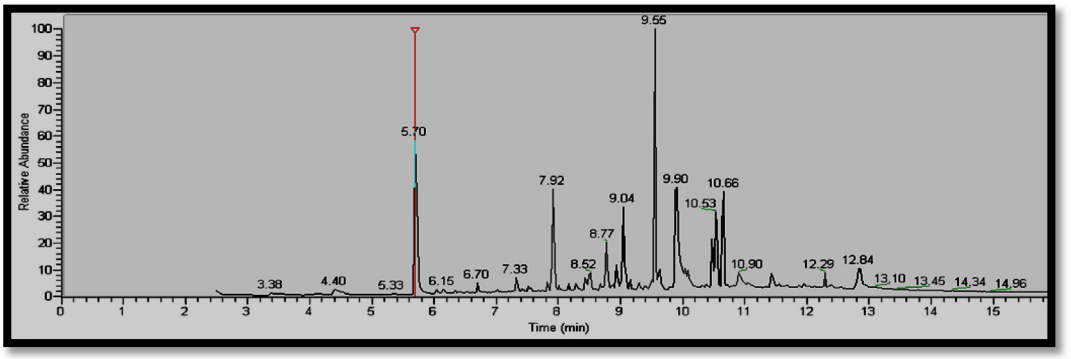

Figure 6 GC/MS chromatogram of *U. fasciata* methanolic extract (UFM1) collected from El-Anfoushy.

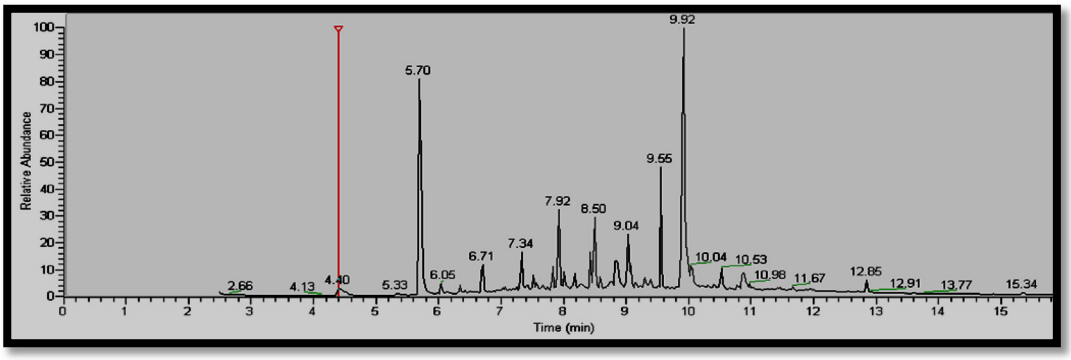

Figure 7 GC/MS chromatogram of *P. capillacea* methanolic extract (PCM1) collected from Abu Qir Bay.

docosanoic acid, (*Z*)-10-hexacosene and isofucosterol (El Ashry et al., 2011). Another study by Abbassy et al. (2014) found that *U. lactuca* methanolic extract contains 42 components and the main five components were 1,2-benzene dicarboxylic acid, bis(2-ethylhexyl) ester, palmitic acid, benzene,1,2,4-trimethyl, 8-octadecanoic acid methyl ester and benzene,1-ethyl-2-methyl.

The methanolic extract of *U. fasciata* (UFM1) was found to contain palmitic acid, methylester (RT = 9.55 min) as the most abundant compound followed by trichloromethyloxirane (RT = 5.70 min), linolenic acid, ethylester (RT = 10.66 min), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (RT = 9.04 min), 11-octadecenoic acid, methylester (RT = 10.53 min) and 12,15-octadecadienoic acid, methylester (RT = 8.77 min) (Table 6). Abou-El-Wafa et al. (2009) found that the dichloromethane extract of the alga furnished the new fatty acids; (*E*)-11-oxo-octadeca-12-enoic acid, 6-hydroxy-oct-7-enoic acid and (*E*)-11-hydroxy-octadeca-12-enoic acid besides cholesterol while the non polar part of the extract produced ten compounds; 4-oxo-pentanoic acid, hexadecanoic acid, dimethylsulfoxide, dimethylsulfone, phenylacetamide, 6,10,14-trimethyl-pentadecan-2-one, 8-heptadecene, dodecane, tridecane, and the new compound 1,1-bicyclohexyl. Sivakumar et al. (2014) reported that crude ethyl acetate extract contains bis (2-ethylhexyl) phthalate and 1,2-benzenedicarboxylic acid- butyl as main chemical constituents. The crude extract of *U. fasciata* has

Table 3 Chemical constituents of *U. lactuca* ethyl acetate extract (ULE2) collected from Al Selsela.

Peak No.	RT (min)	Component name	Molecular formula	MW (m/z)	Hit	SI	RSI	Prob.
1	7.91	Dichloroacetic acid, heptadecyl ester	$C_{19}H_{36}Cl_2O_2$	366	3	737	739	5.39
2	8.28	Unidentified	–	–	–	–	–	–
3	8.79	(9Z)-9,17-Octadecadienal	$C_{18}H_{32}O$	264	4	719	756	4.03
4	9.04	Unidentified	–	–	–	–	–	–
5	9.55	Methyl 14-methylheptadecanoate	$C_{17}H_{34}O_2$	270	1	664	667	14.43
6	9.90	Unidentified	–	–	–	–	–	–
7	10.81	Ethyl oleate	$C_{20}H_{38}O_2$	310	4	719	757	6.69
8	10.92	Oleic acid	$C_{18}H_{34}O_2$	282	1	722	754	14.76
9	11.49	Tributyl acetylcitrate	$C_{20}H_{34}O_8$	402	2	607	672	42.36
10	11.67	Unidentified	–	–	–	–	–	–
11	12.85	Di-n-octylphthalate	$C_{24}H_{38}O_4$	390	1	633	667	11.17

SI = Direct match value; RSI = Reverse match value; Prob. = Probability value.

Table 4 Chemical constituents of *U. lactuca* methanolic extract (ULM5) collected from Al Selsela.

Peak No.	RT (min)	Component name	Molecular formula	MW (m/z)	Hit	SI	RSI	Prob.
1	5.70	*o*-Chlorofluorobenzene	C_6H_4ClF	130	2	618	631	12.53
2	6.15	Unidentified	–	–	–	–	–	–
3	6.70	Decylether	$C_{20}H_{42}O$	289	1	712	746	12.55
4	7.91	1-Heptadecanol	$C_{17}H_{36}O$	256	4	695	716	4.26
5	8.78	3-Icosyne	$C_{20}H_{38}$	278	2	700	716	4.15
6	8.94	Unidentified	–	–	–	–	–	–
7	9.05	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1	718	786	14.36
8	9.55	Oleic acid	$C_{18}H_{34}O_2$	282	5	717	731	4.22
9	9.86	Palmitic acid, ethylester	$C_{18}H_{36}O_2$	284	4	610	702	73.52
10	9.90	Unidentified	–	–	–	–	–	–
11	10.54	10-Octadecenoic acid, methylester	$C_{19}H_{36}O_2$	296	1	771	777	7.83
12	10.82	Ethyl oleate	$C_{20}H_{38}O_2$	310	2	715	734	6.68
13	12.29	Nonadecanoic acid, methylester	$C_{20}H_{40}O_2$	312	3	614	657	7.09
14	12.55	Tridecanoic acid,13-formyl-ethylester	$C_{16}H_{30}O_3$	270	5	588	651	2.37

SI = Direct match value; RSI = Reverse match value; Prob. = Probability value.

Table 5 Chemical constituents of *U. lactuca* methanolic extract (ULM1) collected from Abu Qir Bay.

Peak No.	RT (min)	Component name	Molecular formula	MW (m/z)	Hit	SI	RSI	Prob.
1	5.69	Trichloroethylene	C_2HCl	130	2	558	609	8.54
2	6.04	n-Nonadecane	$C_{19}H_{40}$	268	3	716	786	4.16
3	6.71	7-Methylpentadecane	$C_{16}H_{34}$	226	4	692	725	4.48
4	7.34	n-Octadecyl chloride	$C_{18}H_{37}Cl$	288	3	663	668	6.63
5	7.83	Hexa-hydro-farnesol	$C_{15}H_{32}O$	228	1	690	704	12.70
6	7.94	Myristyl chloride	$C_{14}H_{29}Cl$	232	1	663	682	9.74
7	8.41	Unidentified	–	–	–	–	–	–
8	9.05	Unidentified	–	–	–	–	–	–
9	9.57	Unidentified	–	–	–	–	–	–
10	9.91	Unidentified	–	–	–	–	–	–
11	10.05	6-Methyloctadecane	$C_{19}H_{40}$	268	4	666	737	6.66
12	10.52	5-Octadecenal	$C_{18}H_{34}O$	266	3	714	728	13.09
13	12.85	Phthalic acid, isodecyloctylester	$C_{26}H_{42}O_4$	418	5	702	740	2.70

SI = Direct match value; RSI = Reverse match value; Prob. = Probability value.

been found to contain twenty-eight compounds. The identified compounds include 4-hexahydroxy flavoneacetyl B glucopyranoside, formycin-A, adenosine, 5′-deoxyguanosine and n-alkenylhydroquinol dimethyl ether (Abdel-Khaliq et al., 2014). α-Linolenic acid, stearidonic acid, ulvanobiuronic acid 3-sulfate, bromophenolic, sphingosine-type compound and guaiane sesquiterpene derivatives (guai-2-en-10a-ol and guai-2-en-10a-methanol) have been previously isolated from

Table 6 Chemical constituents of methanolic *U. fasciata* extract (UFM1) collected from El-Anfoushy.

Peak No.	RT (min)	Component name	Molecular formula	MW (m/z)	Hit	SI	RSI	Prob.
1	4.40	Unidentified	–	–	–	–	–	–
2	5.70	Trichloromethyloxirane	$C_3H_3Cl_3O$	160	5	537	538	7.12
3	6.70	3,3,5-Trimethylhexahydroazepine	$C_9H_{19}N$	141	4	648	710	4.39
4	7.33	2-Butyl-1-octanol	$C_{12}H_{26}O$	186	4	682	741	5.97
5	7.92	Unidentified	–	–	–	–	–	–
6	8.52	Unidentified	–	–	–	–	–	–
7	8.77	12,15-Octadecadienoic acid, methylester	$C_{19}H_{34}O_2$	294	4	683	703	4.73
8	9.04	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2	721	800	9.02
9	9.55	Palmitic acid, methylester	$C_{17}H_{34}O_2$	270	1	681	688	38.82
10	9.90	Unidentified	–	–	–	–	–	–
11	10.47	Phytol	$C_{20}H_{40}O$	296	2	718	750	9.79
12	10.53	11-Octadecenoic acid, methylester	$C_{19}H_{36}O_2$	296	2	752	760	6.34
13	10.66	Linolenic acid, ethylester	$C_{20}H_{34}O_2$	306	5	706	711	6.63
14	10.90	2-cis-9-Octadecenyloxylethanol	$C_{20}H_{40}O_2$	312	1	720	737	32.73
15	11.41	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294	5	680	737	3.90
16	12.29	Docosanoic acid, methylester	$C_{23}H_{46}O_2$	354	3	614	639	10.55
17	12.84	Unidentified	–	–	–	–	–	–

SI = Direct match value; RSI = Reverse match value; Prob. = Probability value.

Table 7 Chemical constituents of *P. capillacea* methanolic extract (PCM1) collected from Abu Qir Bay.

Peak No.	RT (min)	Component name	Molecular formula	MW (m/z)	Hit	SI	RSI	Prob.
1	4.40	Bicyclo[2.2.1]heptane-2,3-dithiol	$C_7H_2S_2$	160	1	576	579	13.20
2	5.70	Unidentified	–	–	–	–	–	–
3	6.05	2,5,6-Trimethyldecane	$C_{13}H_{28}$	184	4	723	865	3.51
4	6.71	2-Ethyl-1-decanol	$C_{12}H_{26}O$	186	2	705	754	6.43
5	7.34	Methoxy acetic acid, 2-tridecylester	$C_{16}H_{32}O_3$	272	1	697	742	10.91
6	7.92	n-Heptacosane	$C_{27}H_{56}$	380	2	713	774	7.33
7	8.50	2-Methylhexadecan-1-ol	$C_{17}H_{36}O$	256	1	693	697	11.03
8	8.85	Myristic acid	$C_{14}H_{28}O_2$	228	4	618	648	10.65
9	9.04	Unidentified	–	–	–	–	–	–
10	9.55	Unidentified	–	–	–	–	–	–
11	9.92	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	1	683	683	40.49
12	10.04	Estra-1,3,5(10)trien-17β-ol	$C_{18}H_{24}O$	256	4	663	707	5.21
13	10.53	4-Octadecenal	$C_{18}H_{34}O$	266	3	687	718	8.05
14	10.88	Unidentified	–	–	–	–	–	–
15	12.85	Di-n-octylphthalate	$C_{24}H_{38}O_4$	390	3	542	588	7.35

SI = Direct match value; RSI = Reverse match value; Prob. = Probability value.

U. fasciata (Chakraborty et al., 2010; Paulert et al., 2009). The data recorded for methanolic extract of the red alga *P. capillacea* (PCM1) showed that of the eleven compounds identified; the most prevailing compounds were palmitic acid (n-hexadecanoic acid, RT = 9.92 min), n-heptacosane (RT = 7.92 min), 2-methylhexadecan-1-ol (RT = 8.50 min), methoxy acetic acid, 2-tridecylester (RT = 7.34 min), and myristic acid (RT = 8.85 min) as shown in Table 7. Wefky and Ghobrial (2008) reported that the ethanol extract of the alga afforded hexadecanoic acid, 1,3,6,9b-tetra-azaphenalene-4-cabonitrile,7,9-dibromo-2-(dibromomethyl), 1,3,5,7,9,11-hexavinyl-3,5,9,11, Tris (trimethylsilyl)-8,2′-thioanhyroadenosine, corydaline, 1,3-dimethyl-4-azaphenanthrene, and 1,1,1,3,5,5,5-heptamethyl trisiloxane. However, the acetone extract furnished three compounds only; heptadecane, 4-hydroxy-4-methyl-2-pentanone and 2,3-dimethyl-5-hexan-3-ol. El-Din and El-Ahwany (2015) mentioned that the crude extracts of *P. capillacea* contain n-hexadecanoic acid, cholesterol, heneicosane, tetradecanoic acid, trans-13-octadecenoic acid, *cis*-5,8,11,14,17-eicosapentaenoic acid methyl ester and 1,2-benzenedicarboxylic acid.

Conclusions

Comparison of the chemical composition of the green, red and brown macroalgae collected from various areas of the Egyptian Mediterranean coast of Alexandria showed that, the taxonomic differences and the environment play an important role in the variability of the bioactivity and the chemical constituents. It is important to consider that the algal extracts are unpurified and contain both polar and non polar compounds that could be responsible for the antifungal properties reported here. The unidentified compounds cannot be matched with any

compounds recorded in the library data base. Egyptian marine macroalgae have the ability to produce bioactive compounds of antifungal potential.

Conflict of interest

There is no conflict of interest.

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